

WO 01/12846

PCT/EP00/07736

13

CLAIMS

1. A method for the preparation of metal oxide supports loaded with biomolecules, comprising the steps of :
 - 5 (a) activating the surface of the support by means of a silanating agent comprising an amine group;
 - (b) loading the support by attaching biomolecules to the activated surface, characterized in that subsequently the loaded support is treated with an acidic solution, and provided that the method is not used for the preparation of silica wafers which are aminated
 - 10 by silanation using (3-aminopropyl)monoethoxydimethylsilane and loaded with oligonucleotides.
2. A method for the preparation of metal oxide supports loaded with biomolecules, comprising the steps of :
 - 15 (a) activating the surface of the support by means of a silanating agent comprising an amine group;
 - (b) loading the support by attaching biomolecules to the activated surface, characterized in that subsequently the loaded support is treated with a basic or neutral solution, and provided that the method is not used for derivatization of aluminiumoxide
 - 20 nanoparticles aminated with (3-aminopropyl)triethoxysilane, wherein the basic solution further contains a large excess of N-acetylhomocysteinylactone.
3. The method of claim 1, wherein the solution is of pH 2 to 7 .
- 25 4. The method of claim 3, wherein the biomolecules are oligonucleotides and the pH is 4-5.
5. The method of claim 1, wherein the metal oxide support is a (electrochemically manufactured) porous metal oxide membrane.
- 30 6. The method of claim 5, wherein the metal oxide is aluminium oxide.

WO 01/12846

PCT/EP00/07736

14

7. The method of claim 1, wherein the support is activated by means of a silanating agent comprising an amine group selected from 3-aminopropyltriethoxysilane, 4-aminobutyl-dimethyl-methoxysilane, 3-[2-(2-aminoethylamino)ethylamino]propyl-trimethoxysilane, 3-(2-aminoethylamino)propyl-methyldimethoxysilane, 3-(2-aminoethylamino)propyl-trimethoxysilane, 3-aminopropyl-methyl-diethoxysilane, (3-aminopropyl)tris[2-(2-methoxyethoxy)ethoxy]silane and 4-aminobutyltriethoxysilane,
8. The method of claim 7, wherein the silanating agent comprising an amine group is 3-aminopropyltriethoxysilane.
9. The method of claim 8, wherein 3-aminopropyl triethoxysilane is used in an unbuffered aqueous solution.
10. The method of claim 1, wherein the biomolecules are adsorptively attached to the activated surface of the support.
11. The method of claim 1, wherein the biomolecules are attached to the activated surface in spots, thereby forming an array of spots.
12. The method of claim 11, wherein the biomolecules attached to the surface in different spots may be the same or different.
13. The method of claim 1, wherein the biomolecules are oligonucleotides.
14. A loaded metal oxide support prepared according to the method of claim 1.
15. An aminoalkyltrialkoxysilane-activated metal oxide support, provided with an array of spots of biomolecules attached to the support, characterized in that on the array the density of amino groups on the surface area between the spots is significantly lower than the density of amino groups in the spots.

WO 01/12846

PCT/EP00/07736

15

16. Use of the metal oxide support of claim 14 or 15 for performing a probe-based assay.
17. A kit of parts comprising the metal oxide support of claim 14 or 15, further comprising a
5 detection means for determining whether binding has occurred between the biomolecules
and an analyte.
18. A kit according to claim 17, wherein the detection means is a substance capable of binding
to the analyte and being provided with a label.
- 10 19. A kit according to claim 18, wherein the label is capable of inducing a colour reaction
and/or capable of bio-, chemo- or photoluminescence.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12846 A1

- (51) **International Patent Classification⁷:** C12Q 1/68, [NL/NL]; Fokke, Dode Nieuwstraat 58, NL-5211 Ek's Hertogenbosch (NL).
G01N 33/553
- (21) **International Application Number:** PCT/EP00/07736 (74) **Agent:** HOGENBIRK, M.; P.O. Box 20, NL-5340 BH Oss (NL).
- (22) **International Filing Date:** 9 August 2000 (09.08.2000) (81) **Designated States (national):** AU, CA, ID, JP, KR, US, ZA.
- (25) **Filing Language:** English (84) **Designated States (regional):** European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
- (26) **Publication Language:** English
- (30) **Priority Data:** 99202649.2 16 August 1999 (16.08.1999) EP **Published:**
— With international search report.
— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
- (71) **Applicant (for all designated States except US):** PAM-GENE B.V. [NL/NL]; Grote Gent 2, NL-5261 BT Vught (NL).
- (72) **Inventor; and**
- (75) **Inventor/Applicant (for US only):** VENEMA F.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** PREPARATION OF METAL OXIDE SUPPORTS LOADED WITH BIOMOLECULES

(57) **Abstract:** The invention relates to method for the preparation of metal oxide supports loaded with biomolecules, comprising the steps of: (a) activating the surface of the support by means of a silanating agent comprising an amine group; (b) loading the support by attaching biomolecules to the activated surface, characterized in that subsequently the loaded support is treated with an acidic solution, and provided that the method is not used for the preparation of silica wafers which are aminated by silanation using (3-aminopropyl)monoethoxydimethylsilane and loaded with oligonucleotides. Similarly, an activated and loaded support may be treated with a basic or neutral solution, provided that the method is not used for derivatization of aluminiumoxide nanoparticles aminated with (3-aminopropyl)triethoxysilane, wherein the basic solution further contains a large excess of N-acetylhomocysteinylactone. This method can for example be used in the preparation of arrays for probe-based assays.

WO 01/12846 A1

PTO/SB/01 (10-01)

Approved for use through 10/31/2002. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

**DECLARATION FOR UTILITY OR
DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number 65959/16

First Named Inventor F. Venema

COMPLETE IF KNOWN

Application Number 10/049,804

Filing Date February 14, 2002

Art Unit to be assigned

Examiner Name to be assigned

As the below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original and first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PREPARATION OF METAL OXIDE SUPPORTS LOADED WITH BIOMOLECULES

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 02/14/2002 as United States Application Number or PCT International

Application Number 10/049,804 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
PCT/EP00/07736 EP 99202649.2	PCT	08/09/2000	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
	Europe	08/16/1999	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

[Page 1 of 2]

65959/19

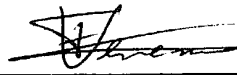
PTO/SB/01 (10-01)

Approved for use through 10/31/2002. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application

Direct all correspondence to: <input checked="" type="checkbox"/> Customer Number OR <input type="checkbox"/> Correspondence address below	
Name <u>Kenneth P. George, Esq.</u>	
Address <u>Amster, Rothstein & Ebenstein</u>	
Address <u>90 Park Avenue</u>	
City <u>New York</u>	State <u>New York</u> ZIP <u>10016</u>
Country <u>USA</u>	Telephone <u>212-697-5995</u> Fax <u>212-286-0854</u>
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.	
NAME OF SOLE OR FIRST INVENTOR: <input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name (first and middle (if any)) <u>F.</u>	Family Name or Surname <u>Venema</u>
Inventor's Signature 	Date <u>17th June 2002</u>
Residence: City <u>'s-Hertogenbosch</u>	State <u>NLX</u> Country <u>Kingdom of the Netherlands</u> Citizenship <u>Kingdom of the Netherlands</u>
Mailing Address <u>Fokke, Dode Nieuwstraat 58, NL-5211 Ek</u>	
City <u>'s-Hertogenbosch</u>	State <u>--</u> ZIP <u>--</u> Country <u>Netherlands</u>
NAME OF SECOND INVENTOR: <input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name (first and middle (if any))	Family Name or Surname
Inventor's Signature	Date
Residence: City	State Country Citizenship
Mailing Address	
City	State ZIP Country
<input type="checkbox"/> Additional inventors are being named on the _____ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.	